#### DOGGGTL DOESCH

#### Constitutively Active Receptors

File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS A GROUP I					
MSHR_mouse	melanocyte-stimulating hormone	TŅII	92 VSIVL <u>E</u> TTIIL K	adenylyl cyclase activity/ HEK293, stably transfected	(Robbins, Nadeau et al. 1993)
	MSH				
CLASS A GROUP II					
5H1B_human	5-hydroxytryptamine <sub>1B</sub>	C-terminus of IC3	313 RERKA <u>T</u> KTLGI K, R, Q	binding of [35S]GTP[S] / CHO-KI	(Pauwels, Gouble et al. 1999)
5H2A_human	5-hydroxytryptamine <sub>2A</sub>	C-terminus of IC3	322 NEQKA <u>C</u> KVLGI K	IP production / COS-7	(Egan, Herrick-Davis et al. 1998)
2H2C_rat	5-hydroxytryptamine <sub>2C</sub>	C-terminus of IC3	312 NEDDA <u>S</u> KVLGI L	PI hydrolysis / COS-7	(Herrick-Davis, Egan et al. 1997)

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CLASS A GROUP II					
A1AB_human	α <sub>18</sub> -adrenergic	TMDI	63 FAIVG <u>N</u> ILVIL	IP / COS-7	(Scheer, Fanelli et al. 1997)
	alpha 1B-AR		A		
•		junction between TMDIII and IC2	142 CAISI <u>D</u> RYIGV A		
A1AB_human	α <sub>19</sub> -adrenergic albha 1B-AR	junction between TMDIII and IC2	143 CAISID <u>R</u> YIGV K	IP/COS-7	(Scheer, Costa et al. 2000)
A1AB_human	α <sub>1B</sub> -adrenergic	TMIII	128 AVDVL <u>C</u> CTASI F	IP/COS-1	(Perez, Hwa et al. 1996)
		carboxyl end of IC3	293 REKKA <u>A</u> KTLGI E	IP arachidonic acid release	
		TMV	204 EEPFY <u>A</u> LFSSLG V	IP/COS-1	(Hwa, Gaivin et al. 1997)
A1AB_human	α <sub>19</sub> -adrenergic	C-terminal IC3	293 SREKKA <u>A</u> KT X=19 different substitutions	PI / COS-7	(Kjelsberg, Cotecchia et al. 1992)
A1AB_human	α <sub>18</sub> -adrenergic	C-terminus IC3	288 293 KFS <u>REK</u> KA <u>A</u> KTLGI K H L	PI hydrolysis / rat fibroblast	(Allen, Lefkowitz et al. 1991)
A2AA_human	α <sub>2</sub> C10-adrenergic alpha-2AAR	C-terminal IC3 loop	373 (348?) EKRF <u>T</u> FVLAV X=F,A,C,E,K	adenylyl cyclase inhibition / HEK293	(Ren, Kurose et al. 1993)
ACM1_human	muscarinic Hm1 muscarinic acetylcholine M1	C-terminal IC3 loop junction	360 SLVK <u>e</u> kkaartls A	PI / HEK(U293)	(Högger, Shockley et al. 1995)
ACM2-human	muscarinic acetylcholine M2	junction of IC3 and TMVI	390 KKVTRTIL†A 1-4 A inserted	IP production, inhibition of cAMP production / COS-7	(Liu, Blin et al. 1996)

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CLASS A					
ACM3_rat	m3 muscarinic (rat)	TMVI	507 TWTPY <u>N</u> IMVLVNT S	IP / COS-7	(Blüml, Mutschler et al. 1994)
ACM5_human	muscarinic acetylcholine M3 m5 muscarinic muscarinic acetylcholine M5	N-terminus to TMII TMVI	chimera composed of m21-69 m577-445	β-gal / NIH 3T3	(Burstein, Spalding et al. 1996)
ACM5_human	m5 muscarinic muscarinic acetylcholine M5	TMVI	M2 391-466 451 459 465 A <u>I</u> LLA <u>F</u> ITTW TPY <u>N</u> I MVLV <u>S</u> T M L C	β-gal; radioligand binding / NIH-3T3	(Spalding, Burstein et al. 1998)
ACM5_human	mscarinic muscarinic muscarinic acetylcholine M5	junction of TMVI and EC3	T 465 YNIMVLV <u>S</u> TFCDKCV X=V,F,R,K,+more	β-gal; radioligand binding / NIH-3T3	(Spalding, Burstein et al. 1997)
B1AR_human	β <sub>1</sub> -adrenergic	C-terminus	389 RKAFQGLLCCA R	adenylyl cyclase; agonist binding / CHW	(Mason, Moore et al. 1999)
B2AR_human	β <sub>2</sub> -adrenergic beta-2AR	C-terminal IC3 loop	266 272 FC <u>LKEH</u> KA <u>L</u> KTLGI SR K A	adenylyl cyclase activation; agonist binding affinity / COS-7 or CHO	(Samama, Cotecchia et al. 1993); (Lefkowitz, Cotecchia et al. 1993)
DADR_human	dopamine D1A	carboxyl terminal IC3	264 SFKMSEKRETKVLKT I K 288 from DIB receptor APDTSIKKETKVLKT	adenylyl cyclase; cAMP accumulation / HEK293	(Charpentier, Jarvie et al. 1996)
DADR_human	dopamine D1	TMVI	286 FVCCW <u>L</u> PFFIL A	CAMP accumulation / COS-7	(Cho, Taylor et al. 1996)
HH2R_rat	histamine H <sub>2</sub>	IC2	115 FMISL <u>D</u> RYCAV N,A	cAMP production / HEK-293	(Alewijnse, Timmerman et al. 2000)

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File Name	Recentor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS A					
OPSD_human	opsin	TMII	90 FMVLG <u>G</u> FTSTLY	transducin; phosphorylation by	(Rim and Oprian 1995)
	rhodopsin	TMIII	113	rhodopsin kinase / CUS	
		TMVII	GCNJEGFFAT . Q 292 296 MTIPAPPAKSAAIY		
			E G, E, M  292 Ala neutral a.a converted to carboxylate and competes with <sup>113</sup> Glu for salt bridge with <sup>296</sup> Lys		
OPSD_human	opsin	TMIII	134 VVLAIERYVVV	transducin; radioligand binding / COS	(Acharya and Karnik 1996)
	rhodopsin		s,0,1	5	
OPSD_human	opsin	TM6	257 RMVIIMVIAFL	transducin, GTP $\gamma$ S uptake / COS	(Han, Smith et al. 1998)
	rhodopsin		N'A	•	
		plus TM3	plus G113Q		
OPSD_human	opsin	TMVII	296 PAFFA <u>K</u> SAAIY	transducin; radioligand binding / COS	(Govardhan and Oprian 1994);
	rhodopsin		G X=E,M natural mutants + 10 different a.a. substitutions		(Cohen, Yang et al. 1993)
			disrupts critical salt bridge between 296Lys(TMVII) and 113Glu(TMIII)		
		1C2	134 VVLAL <u>E</u> RYVVV Q		(Cohen, Yang et al. 1993)

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TRFR mouse	thyrotropin-releasing hormone   carboxyl tail	carboxyl tail	335	<sup>45</sup> Ca <sup>2+</sup> efflux, [Ca <sup>2+</sup> ] /	(Matus-Leibovitch,
	TRH-R	•	FRKLCNCKOK	Xenopus oocytes;	Nussenzveig et al. 1995)
			STOP	IP formation / AtT20,	
				stably transfected	

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#### Figure '1 (Page 6 of 15)

		W 4 - 4 - 5 0 54 -	Cognonce	Assav / Cells	Reference
File Name	Receptor	Mutation Site	action and		The state of the s
CLASS A					
AG2R_rat	ΑΤιλ	TMIII	111 ASVSF <u>N</u> LYASV	phospholipase C; IP production / COS-7	(Groblewski, Maigret et al. 1997)
	Type-1A angiotensis II		A disrupts <sup>111</sup> Asn(TMIII)- <sup>292</sup> Tyr(TMVII) interaction		
AG2R_rat	ΑΤι <sub>Α</sub>	C-terminus of TM7	305 LFYGF <u>L</u> GKKFK	IP production / HEK-293; intrcellular Ca <sup>2*</sup>	(Parnot, Bardin et al. 2000)
	Type-1A angiotensis II	other multiple mutations	51	PI production;	(Amatruda, Dragas-
FMLR_human	formylmethionylleucyipnenylai anine (fMLPR)		LVIWVAGFRMTHTVTTISYLNKAVA LVVWVTAFEAKRTINAIWFLNLAVA	phospholipase C stimulation / COS-7	Graonic et al. 1995)
			(K above contince with SWISS-PROT database)		
IL8B_human	interleukin-8 receptor B	IC2	138 ACISV <u>D</u> RYLAIVH	IP production; Ca <sup>2+</sup> moblization and actin	(Burger, Burger et al. 1999)
	CXCR-2 chemokine		Λ	polymerization / NIH 3T3	
LSHR_human	luteinizing hormone (LH)	103	564 MATNK <u>D</u> TKIAKK G	cAMP production / HEK293	(Kudo, Osuga et al. 1996)
LSHR_human	luteinizing hormone (LH)	TMVI	578 ILIFT <u>D</u> FTCMA G	cAMP production / COS-7	(Shenker, Laue et al. 1993)
LSHR_human	luteinizing hormone (LH)	TM6	571 577 KIAKKMAILIFIDFTCM I I	cAMP production / COS-7	(Kosugi, Van Dop et al. 1995)
LSHR_rat	luteinizing hormone / human chorionic gonadotropin	TMVI	556 ILIFT <u>D</u> FTCMA G, Y	cAMP production / HEK 293T	(Bradbury, Kawate et al. 1997; Bradbury and Menon 1999)
OPRD_mouse	delta opiod receptor	TM3	128 KVLSI <u>D</u> YYNMF A, K, H	adenylyl cyclase inhibition / COS-7	(Cavallı, Babey et al. 1999)
OXYR_human	oxytocin	IC2	137 LMSLDRCLAIC A	IP production / COS-7	(Fanelli, Barbier et al. 1999)

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PAFR_human	platelet-activating factor (PAF)	C-terminus of IC3	231 231 231 231	IP production / COS-7	(Parent, Le Gouill et al.
			EVNKKA <u>u</u> minvoiviav R		. (986)
PAFR_human	platelet-activating factor (PAF)	TMIII	100 CLFFI <u>N</u> TYCSV A	arachnidonate release, IP production, adenylyl cylcase inhibition / CHO	(Ishii, Izumi et al. 1997)
PE23_human	prostaglandin E <sub>3</sub> , EP3III EP3IV	C-terminal tail	360 FCQ <u>EEFWGN</u> FCQMRKRRLREQEEFWGN ^ Lruncated	inhibition of adenylyl cyclase / CHO-K1	(Jin, Mao et al. 1997)
PE23_mouse	prostaglandin E, EP3	carboxyl-terminal tail	336 KILLRKFCQ <u>IRDHT</u> (3α) <u>MMNHL</u> (3β) †truncated	inhibition of adenylate cyclase / CHO, stably expressed	(Hasegawa, Negishi et al. 1996)
THRR_human	thrombin	EC2 loop	259 268 CHDVI <u>NETLLEGYYA</u> YY DLKD KDF I	<sup>45</sup> Ca <sup>2*</sup> efflux, PI hydrolysis, reporter gene induction / COS-7	(Nanevicz, Wang et al. 1996)
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	BC1	486 YYNHA <u>I</u> DWQTG F,M	inositol phosphate diacylglycerol cascade / COS-7	(Parma, Van Sande et al. 1995)
		EC2	568 YAKUS <u>I</u> CLPMD T		
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	TMIII	509 ASELS <u>V</u> YILTV A	adenylyl cyclase activation / COS-7	(Duprez, Parma et al. 1994)
		TMVII	672 YPLNS <u>C</u> ANPFL Y		
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	TMV	597 VAFVI $\underline{V}$ CCCHV L	cAMP formation / COS-7 cells	(Esapa, Duprez et al. 1999)
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	TMVII	677 CANPF <u>L</u> YAIFT V	cAMP formation / CHQ cells	(Russo, Wong et al. 1999)
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	IC3	613 621 VRNP <u>OYNPGDKDTK</u> IAK deletion	cAMP formation / COS-7	(Wonerow, Schoneberg et al. 1998)

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TSHR_human	TSHR_human thyrotropin (TSHR)	IC3 / TMVI	623 632 KDTKI <u>A</u> KRMAVLIF <u>T</u> DFICM V	cAMP activation / COS-7	(Paschke, Tonacchera et al. 1994)
	thyroid stimulating nominone			, ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	(1000)
V2R_human	vasopressin V2	IC2	136 LAMTL <u>D</u> RHRAI	CAMP Iornation / COS-7	(Morin, Cotte et al. 1990)
			¥		

File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS B GROUP I					
CALR_human	human calcitonin hCTR-1 hCTR-2	wild type (native) protein		adenylyl cyclase cAMP production / COS-1	(Cohen, Thaw et al. 1997)
CLASS B GROUP II					
PTRR_human	parathyroid hormone PTH / PTH-related peptide	junction of IC1 and TMII	223 TRNYI <u>H</u> MHLFL R, K	cAMP accumulation / COS-7	(Schipani, Jensen et al. 1997)
		junction of IC3 and TMVI	410 KLLKS <u>T</u> LVLMP C,others		
CLASS B GROUP III					
GIPR_human	glucose-dependent insulinotropic peptide (GIP-R)	TMVI	340 VFAPV <u>T</u> EEQAR P	cAMP production / L293	(Tseng and Lin 1997)
GLR_rat	glucagon	junction of IC loop1 and TMII	178 TRNYI <u>H</u> GNLFA R	cAMP accumulation / COS-7	(Hjorth, Orskov et al. 1998)
		IC end of TMVI	352 RLARS <u>T</u> LTLIP A	1	
VIPR_human	vasoactive intestinal peptide 1 (VIP)	junction of IC loop 1 and TMII	178 RNYI <u>H</u> MHLFI R requires functional integrity of the N-terminal EC domain	cAMP production / COS-7 or CHO	(Gaudin, Maoret et al. 1998) (Gaudin, Rouyer-Fessard et al. 1998)
		junction of IC loop 3 and TMVI	343 LARS <u>T</u> LLLIP X= K,P		

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Tella Mana	Docutor	Mutation Site	Sequence	Assay / Cells	Reference	
rile Name	Receptor	Itananon Dite	2000			,
CLASS C			THE CONCURS AND ADDRESS OF THE PERSON OF THE	W / 1. A	(Toncon Cnolding at al	
CASR_human calcium-sen	calcium-sensing	N-terminal EC	TLSEVA <u>ONAL DELA LUBER CANCED IN</u> various substitutions, in multiple combinations	IF / USA	(Jonson, Spanding et al.	
			•			
						٠,
						1

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File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS D					
O74283 RCB2	pheromone	TM6	229 PLSAYQIYLGT P	heterologous yeast assay	(Olesnicky, Brown et al. 1999)
STE2_yeast	pheromone α-factor	TM6	258 QSLLV <u>PS</u> IIFI LL	lacZ reporter gene	(Konopka, Margarit et al. 1996)
STE2_yeast	pheromone α-factor	double mutations TM5	223 MSFVL <u>V</u> VX∰ILAIR C C C	<i>lacZ</i> reporter gene / yeast	(Dube, DeCostanzo et al. 2000)
		TM6	DSFHILLIMGCOSLL CC CC		
			double mutations		
STE3_yeast	pheromone a-factor	IC3	194 DVRDI <u>L</u> HCTNS Q	β-galactosidase	(Boone, Davis et al. 1993)
STE2_yeast	pheromone α-factor	TM6	253 258 LIMSC <u>O</u> SLLV <u>PS</u> IIFI L LP	β-galactosidase	(Sommers, Martin et al. 2000)

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Light Emission Induced by the WT CCK-BR vs. a Constitutively Active Mutant

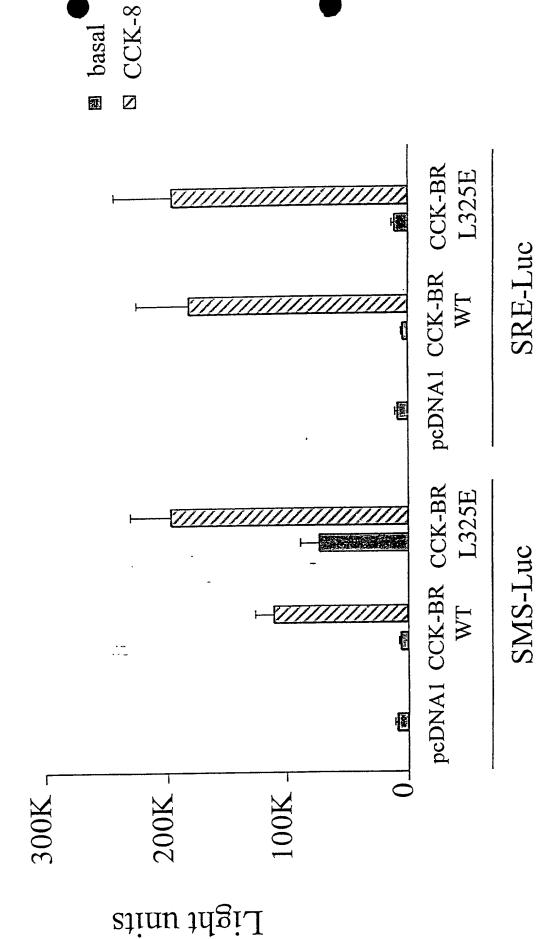


Figure 2

# A Point Mutation Confers Constitutive Activity to the Rat µ Opiod Receptor

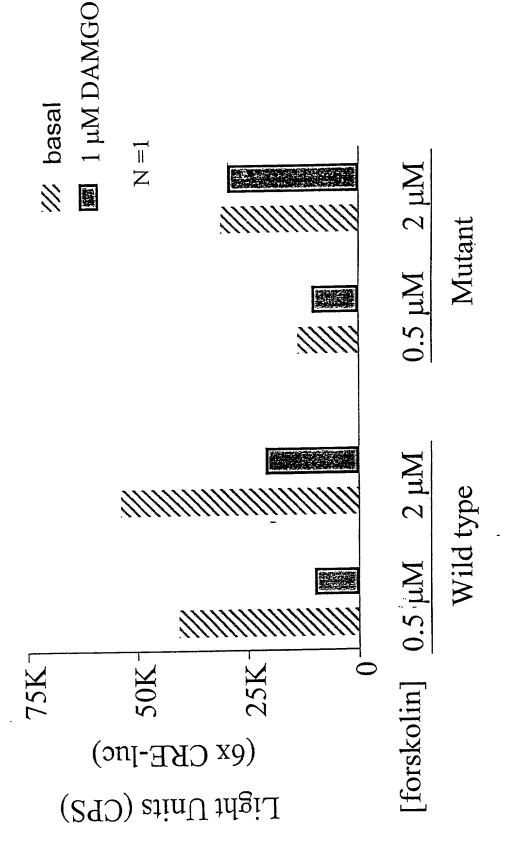


Figure 3

Forskolin Stimulated HEK293 Cells Transfected With pcDNA1 and a CRE-luc Construct

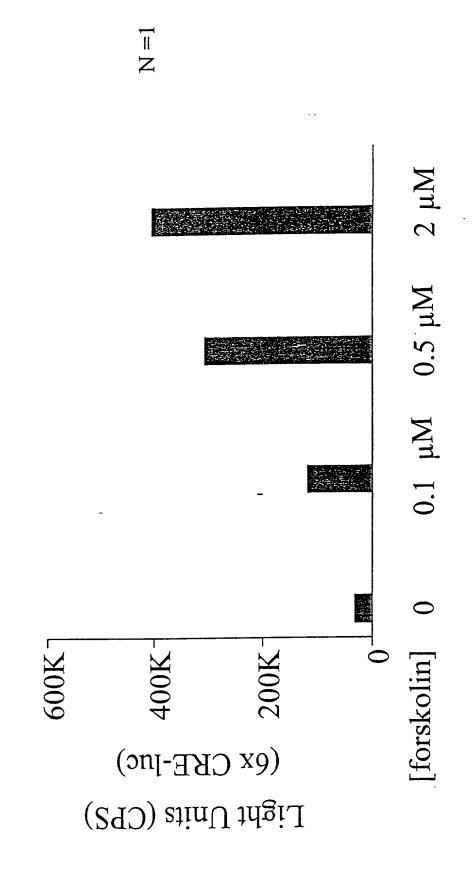


Figure 4

# The Rat $\mu$ Opioid Receptor Signals Through $G\alpha i$

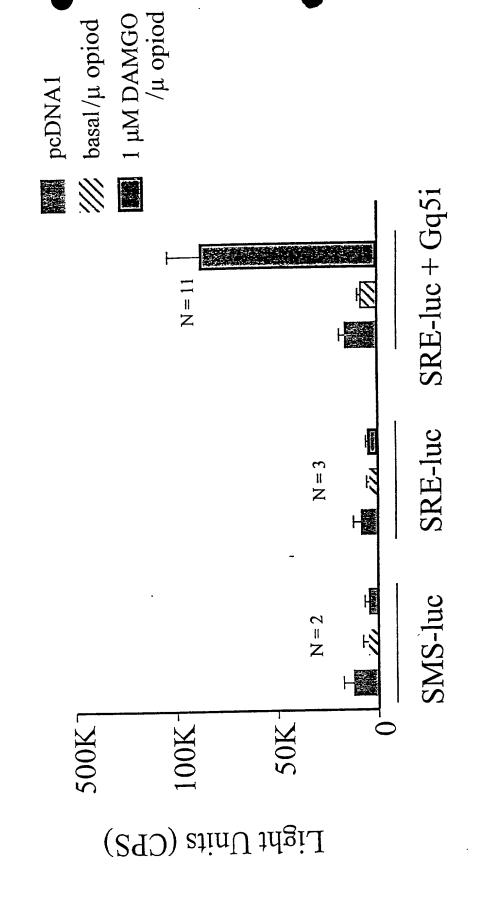


Figure 5

# A Point Mutation Confers Constitutive Activity to the Rat $\mu$ Opioid Receptor

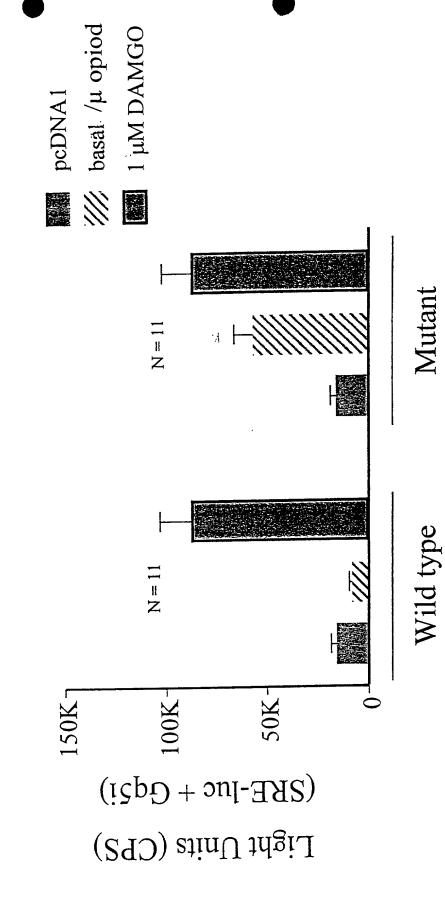


Figure 6

# Target Residues Within Class I GPCRs

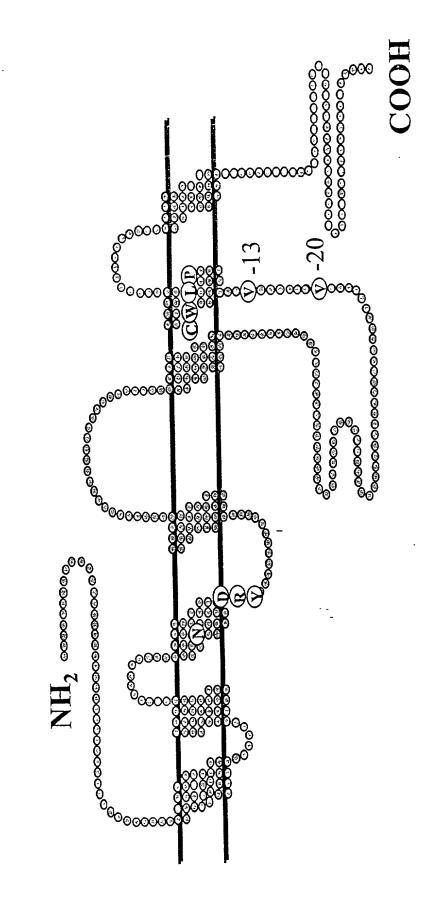


Figure 7

# for Mutation Induced Constitutive Activity TMD III Asn (-14 from DRY) is a Target

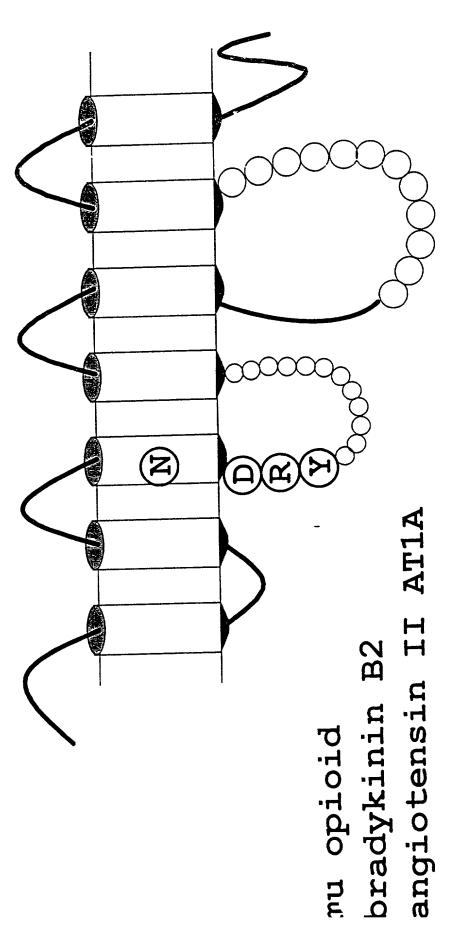


Figure 8

## The 'DRY' Motif is a Target for Mutation Induced Constitutive Activity

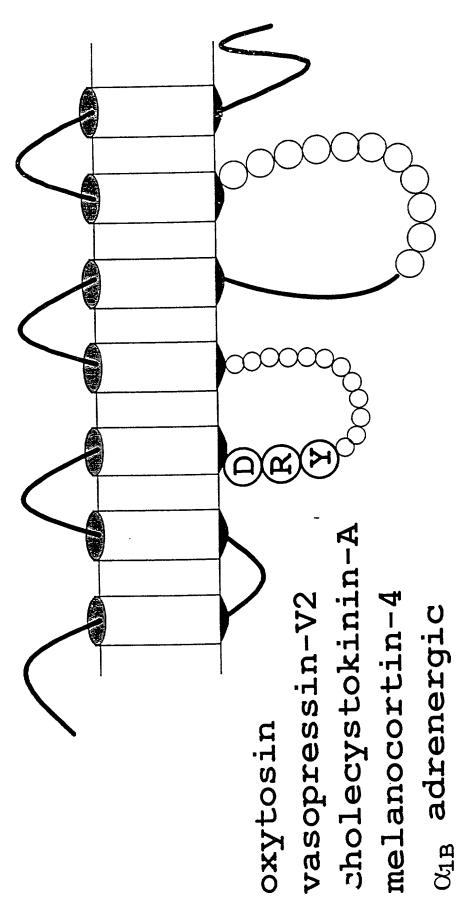
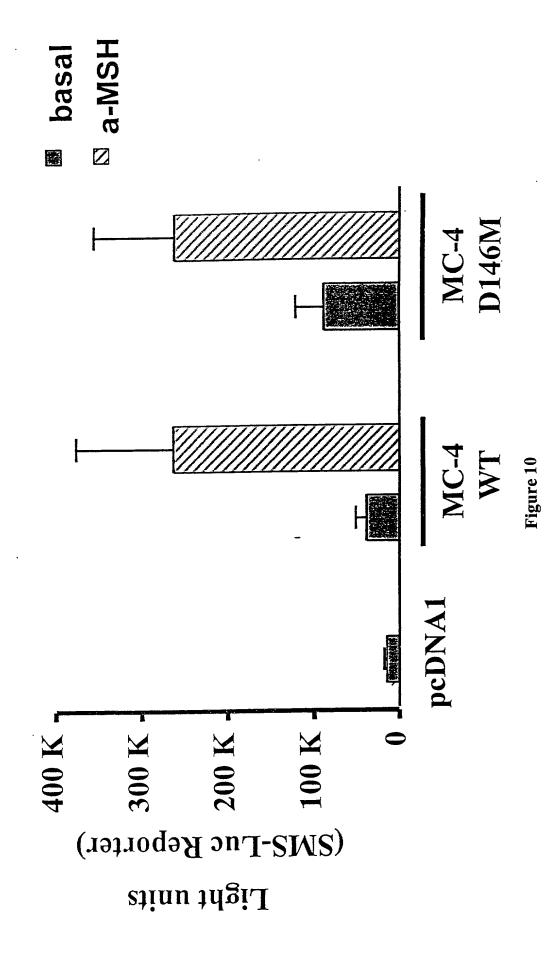


Figure (

A Point Mutation Enhances MC-4 Receptor Constitutive Activity



## The -13 Position is a Target for Mutation Induced Constitutive Activity

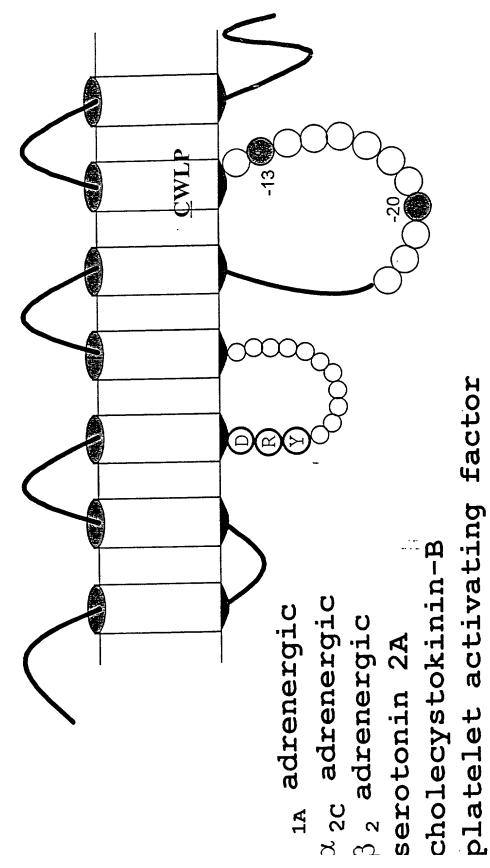


Figure 11

thyroid stimulating hormone

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ork
     orkr
                             1 -----malnssaedcikrio
1 -----mfspwkismflsvredsvpttosfsodmlnvtlogptlng.tfao
    ATla
                     49 LEPAHISPAH. PVHITANYSIVEVVGINGNS LVMEVINRYTKMKTATMIYIFMLALADA
49 LEPAHISBAH. PÜÜİTANYSIVEVVGINGNSIVVHVIRRYTKMKTATMIYIFMLALADA
59 CPPTGS. PSMITAITIMALYSIVCVVGIFGNFLWUMVINRYTKMKTATMIYIFMLALADA
57 CPQTGS. PSMVTAHTIMALYSIVCVVGIFGNFLWUMVINRYTKMKTATMIYIFMLALADA
37 PPGARSASSÜALARA ITALYSAVCAVGIA GAVLUWÜĞILEN TKUKLATMIYIFM ALADA
16 DDCPRAGRHSYIFWÜIPTLYSIEFFVCHFGNSIVÜIVIYFYMKIKIVASÜFLINLALADL
45 SKCPQVEWLGWLNT OPPFLWWEFVENTENIFVESVFCLHKSSCTVAE
     ork
     AT1a
    BK-2
 ork 107 IVIERTMEPFOSTVYLMI SWEPFGERICKIVISIDYYNWFTSIFTLIGMSVDRYIAVCHPVK
orkr 107 IVIERTMEPFOSAVYLMI SWEPFGERICKIVISIDYYNWFTSIFTLIGMSVDRYIAVCHPVK
orm 118 IAISTLEFOSMAVLMG RWEPFGERICKIVISIDYYNWFTSIFTLIGMSVDRYIAVCHPVK
ord 97 IAISTLEFOSMAVLMG RWEPFGERICKIVISIDYYNWFTSIFTLIGMSVDRYIAVCHPVK
ord 97 IAISTLEFOSAKYLME RWEPFGERICKAVISIDYYNWFTSIFTLIMMSVDRYIAVCHPVK
AT1a 76 CFLLTIPLWAYYTAMEYRWEPFGHLCKIASASVTENIYASVELLICISTDRYKATVHPMK
BK-2 105 ILACGLEFFWARTISNNFDWLFGETLORGVNARIISMNFYSSICFUMLVSEDRYMALVKTMS
                                                                                                                                                                          - 14 from DRY
ork 166 ALDERTELKAKI INICIWILSSSIGH SANVIGGIKVR. BDVDVIECSLOFEDDDYSWWD ork 166 ALDERTELKAKI INICIWILSSSIGH SANVIGGIKVR. BDVDVIECSLOFEDDDYSWWD orm 177 ALDERTERNAKI INICIWILSSAIGH PWWFWATIKYR. Q. GSIDCHLTESHPTW. YWE ord 175 ALDERTERNAKI INICIWALSSAIGH PWWFWATIKYR. Q. GSIDCHLTESHPTW. YWE ord 156 ALDERTERNAKI INICIWALSSAIGH PWWFWATIKYR. Q. GSIICHLTESHPTW. YWE ATIA 136 SRLRRIMLVAKWICIII WWAGLASH PAVHHRNV. YFIBNINI IVOAFHYESRN. STLP BK-2 165 MGRMRGVRWAKWYSIVI WGCILLISSPWHVFRIMKEYSDBGHNVIACVISYDS. . . LIWE
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ORM 232 NLDKI CVELFAFIL PVLI LIVCYGLMI LRLKSVRILLSGSKEKDRNLRRI TRLVLVVVAVF
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ORM 211 TVTKI CVELFAFIL PVLI LIVCYGLMI LRLKSVRILLSGSKEKDRSLRRI TRLVLVVVAVF
AT1a 193 IGIGITKNI LGELFEFLI LITSYTLIWKALKKAYE IQKNIKPRIDD... IFRA I MAŽIVLEF
BK-2 222 VFTNIJL DNVJERI P. LSVI TFOTVO I MOVLRNNEMOKEKE IQTE. RRA I VLIVLVI LIGE
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orm 290 TVCWTPIHITYVEIKALUTIP ENTFOTVSWHFCIALGYTNSCLNPVLYAFLDENF
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Figure 12

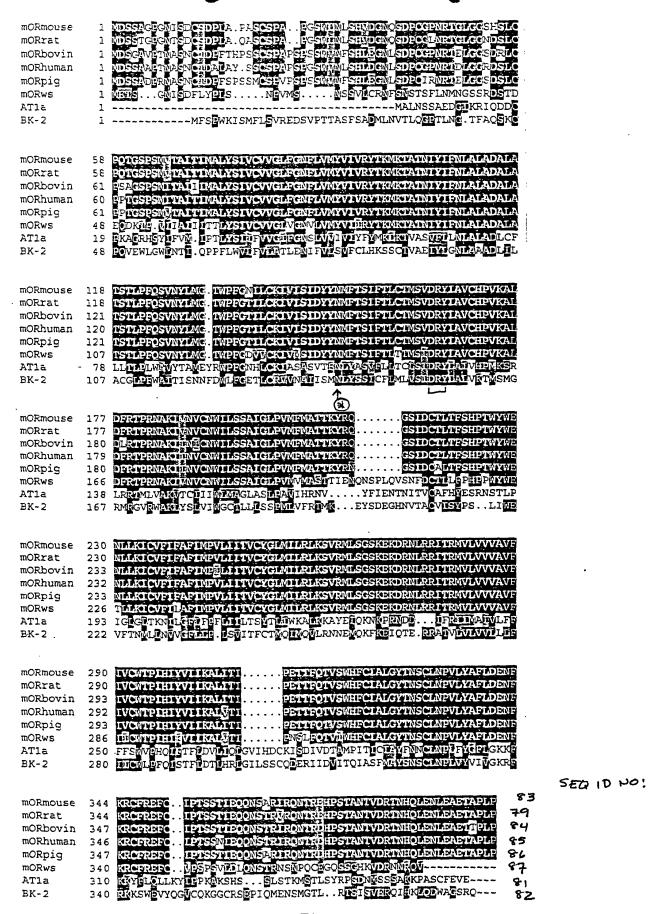


Figure 13